Acta Pharmaceutica Turcica
46 :83 - 94 (2004)

Determination of LAS by Metachromatic Method in Seawater and Reservoir Water

LAS'ın Metakromatik Metod ile Deniz Suyunda ve Havza Suyunda Tayini

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Abstract

Linear alkylbenzene sulfonate was determined usually by methylene blue active substances method (MBAS) in water at α-band of the dye. A metachromatic method is proposed for the determination of anion active substances using various dyes as methylene blue, azur I, Nile blue and toluidine blue at α- and β- bands. Similar results were obtained with MBAS and metachromatic method.
Thus the proposed metachromatic method can be used as well as MBAS method.

Key Words: LAS, metachromasy, methylene blue, azure I, Nile blue, toluidine blue.

Introduction

Detergent is a cleansing agent. It is composed of anionic surfactants (20%), silicates, phosphates, sodium sulfate and others (builders and suspension agents).
Anionic surfactants are divided basically into three groups.

1. Carboxylic acid derivates as fatty acid salts, soaps, amide linked carboxylic acids.
2. Sulfuric ester derivates as alcohol esters, olefin sulfates, ester-and ether-linked sulfonates, alkylaryl sulfonates,alkane sulfonates,
3. Other anionic surfactants are phosphorus compounds (R_2PO_4H, RPO_4H_2), anionic resin derivatives and lignin derivates.

Various builders prossessing inorganic and organic groups were used in the detergents. Inorganic builders or additives in surfactants are: cleaners; caustic soda for glassware, sodium metasilicate and trisodium phosphate for ceramic surface, sodium pyrophosphate for metals. The condensed phosphates, pyrophosphates ($Na_4P_2O_7$), triphosphates (tripolyphosphates $Na_5P_3O_{10}$), glassy phosphates ($Na_6P_6O_{18}$) and metaphosphates $(NaPO_3)_n$ are frequently used in detergent formulations. Organic builders increase the surface activity of detergents. These groups include oil-suspending agents, sodium carboxymethylcellulose, anti-redeposition agents, sequestering agents, ethylenediaminetetraacetic acid (EDTA) and nitriloacetic acid (organic chelating agents) for heavy metal ions. In addition, foam stabilizer and promoter lauryl sulphate is used in a small proportion in detergents.

Sequestering agents for heavy metal cations and silicates, orthosilicates (Na_4SiO_4), metasilicates (Na_2SiO_3) and sesquisilicates are less alkaline and act as inhibitors and reduce the corrosive action of the sulphated anionic detergents on aluminium, brass, potmetal and other metals.

Among the anionic surfactants, linear alkylbenzene sulfonate (LAS) is a major component of detergents. LAS is a complex mixture of C_8-C_{16} alkylbenzene sulfonate. It is prepared by sulfonation of linear alkylbenzene (LAB). It conents paraffin, <5 phenyl $C_{10,}$ phenyl C_{12}, phenyl $C_{13,}$ phenyl C_{13+} phenyl C_{14}, total linear alkylbenzene, total branched alkylbenzene. Beside these elements it contains 2 phenyl alkanes, tetralins and indans. Total amounts of linear alkylbenzene and branched alkylbenzene were 95-96 and 3-4% successively. The main problem was the sulfonation form of benzene ring of LAB. During the process oxydation occurs, polimerization and decomposition of the molecule take place. The little amount of LAB remains in unsulfonated form. After sulfonation various isomers are produced thus indicating that LAS contains many derivatives. Its determination in water is of prime importance for monitoring pollutions. The surfactant in the sewage causes the formation of stable foam and decrease the settling of solid matter. 5 ppm of a surfactant can cause foaming problem in a stream or at a sewage plant. Silicons are very effective to reduce foaming. Surfactant in 2 ppm concentration can cause a perceptible taste in water.
The toxicity and biodegradation of LAS are very important in life and environment. It is toxic for marine life since it decreases oxygen content of water. 1 to 20 ppm surfactant is fatal to species of fish (Weith and Konasewich, 1975; Spehar *et al.*, 1979). Its level of 5 ppm destroyes epithelial gill cells of fish (Ecbert *et al.*, 1974). LAS toxicity was investigated on the effect on plasma parameters (Koç *et al.*, 2001) and lethal concentration and accumulation in gill, heart, stomach and liver on Rainbow trout (*Oncorhynchus myskiss*) (Koç and Güven, 2002).

LAS is a more rapid biodegradable compound. This property is preferable and suitable for prevention of pollution of seas. It is not stable in water and degraded by ω-oxidation of terminal methyl group through the alcohol, aldehyde to be carboxylic acid. It is followed by β-oxidation to a series of short chain sulfophenyl carboxylic acids as sulfophenyl butanoic acid and sulfophenyl pentanoic acid (Black and Howes, 1980). Various types of microorganims were found to affect the degradation of LAS. Microbial oxidation results are phenylacetic, fumaric and acetoacetic acids and benzene ring can be converted to catechol (Hashim *et al.*, 1992). LAS degraded in 20 days 84.8% in distilled water, 14 days 84.8% in tap water and in 14 days 93.2% in seawater (Koç *et al.*, 2002).

The determination of LAS was made by various methods such as: potentiometric, spectrophotometric as methylene blue active subtance (MBAS) (Standard Methods, 1995). Metachromatic (Güven *et al.*, 1994; Akıncı *et al.*, 1997), IR (Helmann, 1978), AAS (Crips *et al.*, 1976; Barrais *et al.*, 1984), GC/MS (Hon. Nami *et al.*, 1978,1980; Eganhouse *et al.*, 1983; Raymundo and Preston, 1992), HPLC (Marcomini and Giger, 1987; Terzic and Ahel, 1993; Koç *et al.*, 2002).

MBAS method is based on the formation of an ion pair product of methylene blue with anion active substances. This complex is blue coloured, soluble in chloroform and its absorbance is measured in a spectrophotometer at 652 nm.

Metachromasy was first found by Ehrlich (1887) in the histological staining of particular tissue elements. This phenomenon was elucidated by Lison (1935), it based on the change of the absorption band (α-band) of the dyes from long to shorter wavelengths (ß- bands). Metachromasy was first used for determination of LAS (Güven *et al.*, 1994; Akıncı and Güven, 1997) and sodium lauryl sulfate (Akıncı and Güven, 1998) and for identification of LAS by thin layer chromatography (Akıncı and Güven, 1992) as well.
Many compounds such as mucopolysaccharides and nucleic acids produced by organisms and the substances as CMC, silicates, polyphosphates, and some ions (salts) added to detergents interfere with the MBAS and metachromatic assay and the results vary depending on the pollution.

In this work the determination of LAS was examined in sea of Marmara and Ömerli reservoir water by using metachromatic dyes as methylene blue, azur I, Nile blue, toluidine blue at α-, ß- bands.

Materials and Methods

LAS (99.7 %) was obtained from Lever Co., Detergent Manufacturing Plant, Gebze, Izmit, Turkey.
Stock LAS solution: 0.100 g LAS (calculated on active substance) in 100 ml distilled water.
Standard LAS solution: 1 ml stock solution was adjusted to 100 ml in distilled water (1 ml =10µg LAS) and prepared daily.
Phenolphthalein solution (Merck): 1/100 in alcohol*.
Sodium hydroxide(Merck): 1N NaOH*.
Sulfuric acid(Merck): 1N H_2SO_4 and 6N H_2SO_4*.
Wash solution (Back Wash): 6.8 ml. conc. H_2SO_4 in 500 ml distilled water, added 50 g $NaH_2PO_4.H_2O$ and the volume was adjusted to 1000 ml*.
Water samples were taken into 3L dark glass bottles from Yenikapı (Sea of Marmara) and Ömerli Reservoir.

* These solutions were prepared according to Standard Methods 1995.

Dye Solutions:

1-Methylene blue(MB) (Merck): 100 mg methylene blue was dissolved in 100 ml distilled water, 30 ml was taken and added 500 ml distilled water and 6.8 ml conc. H_2SO_4 and 50 g sodium dihydrogene phosphate monohydrate, then the volume was adjusted to 1000 ml with distilled water.

2-Azur I (Merck)/ Toluidine blue (Fluka):
100 mg azur I/ toluidine blue was dissolved in 100 ml water.

3-Nile blue: 10 mg Nile blue was dissolved in 50 ml distilled water.

3.1. Nile blue in barbiton buffer: 2.76 g barbiton was dissolved in 25 ml of 0.5 N NaOH and added 7.5 ml Nile blue solution (indicated above) then the volume adjusted to 250 ml with distilled water.

Method

1.*Calibration curve*

The calibration curve was plotted for LAS in conc. of 20-100µg/L with tested dyes.
20, 50, 100µg/L LAS added to 800 ml distilled water in a separatory funnel and added 5 drops of phenolphthalein solution. Make the solution alkaline by dropwise addition of 0.1 N NaOH. Discharge the pink color by dropwise addition of 0.1 N H_2SO_4. Add 30 ml chloroform and 25 ml tested dye solutions. It was extracted 3 times with 30ml chloroform. The chloroform phases were combined and shaken with the wash solution then filtered through glass wool and the volume was adjusted to 100 ml with chloroform. The calibration curve was plotted by using a spectrophotometer (Shimadzu UV 1601) at α- and β- bands their equations were taken from apparatus.

2. Determination of the anionic detergent (LAS) in seawater and Ömerli reservoir.
800 ml samples were used and LAS was determined with the method as described above.

Blank was run with chloroform omitting LAS in the assay.

25- 50 µg LAS was added in the some samples for the control of the assays.

Results and Discussion

The α – and β- bands of dyes are shown in Table 1.

Table 1. α- and β-bands of tested metachromatic dyes.

Dyes	λ max (α-band) (nm)	Metachromatic band β-band (nm)
Methylene blue	652	600
Azur 1	652	620
Nile blue	627	560
Toluidine blue	655	625

The spectrum of LAS with metachromatic dyes are shown in Figs (1 – 4)

Fig. 1. The spectrum of methylene blue with LAS

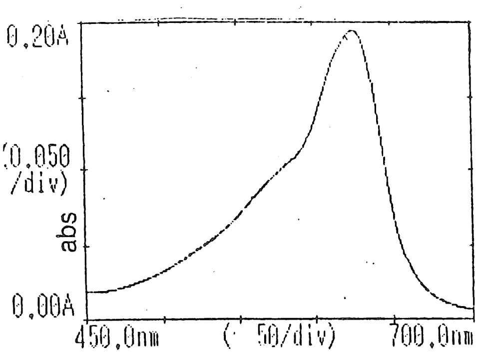


Fig. 3. The spectrum of Nile blue with LAS

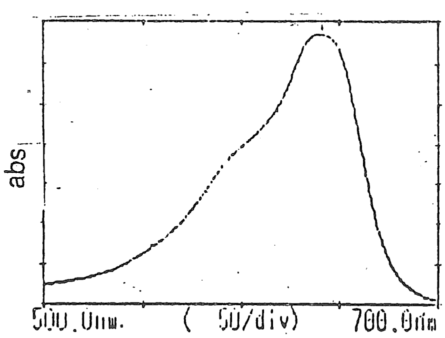


Fig. 2. The spectrum of azur 1 with LAS

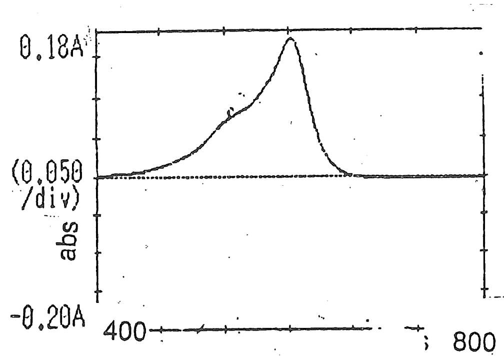
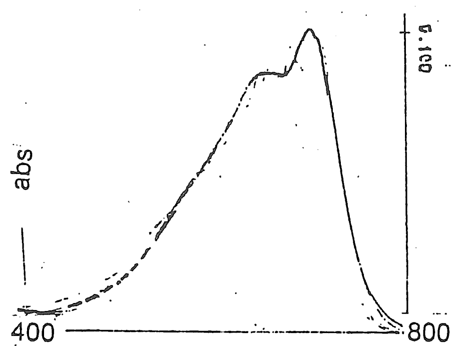


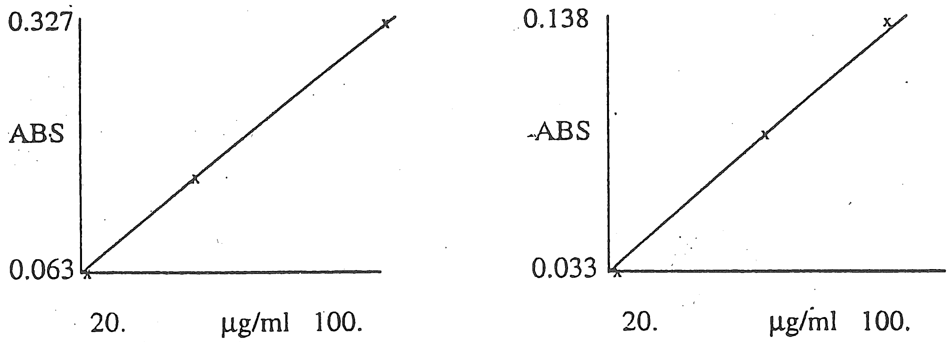
Fig. 4. The spectrum of toluidine blue with LAS



The standard curve of the examined dyes at α- and β- bands are shown in Figs. 5-8.

Fig. 5. The standard curve and its equation of methylene blue with LAS.

a) at 652 nm — b) at 600 nm



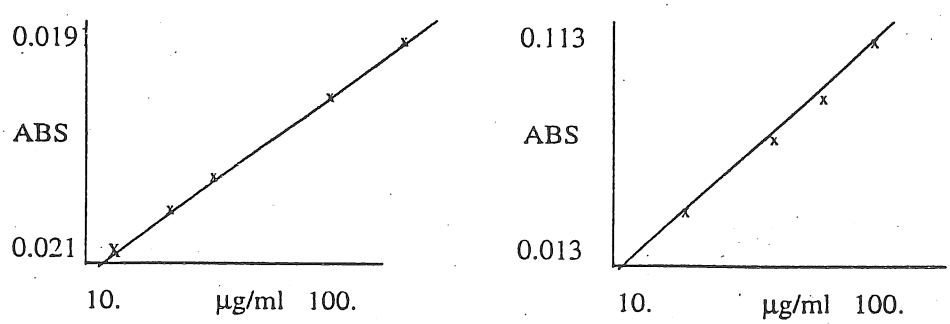
$$ABS = K3C^3 + K2C^2 + K1C + K0$$

a) $K1 = 0.0033$; $K0 = -0.0047$; $r^2 = 0.9998$

b) $K1 = 0.0013$; $K0 = 0.0069$; $r^2 = 1.0000$

Fig. 6. Standard curve and its equation of azur I with LAS.

a)at 640 nm — b) at 620 nm



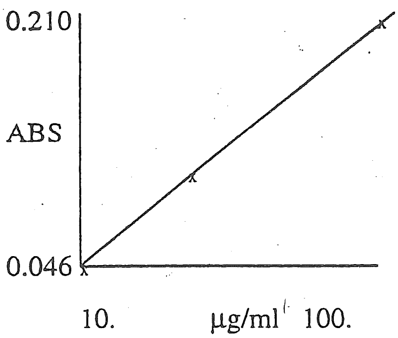
$$ABS = K3C^3 + K2C^2 + K1C + K0$$

a) $K1 = 0.0018$; $K0 = - 0.0090$; $r^2 = 0.9800$

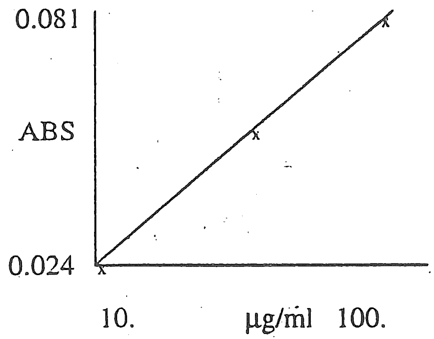
b) $K1 = 0.0011$; $K0 = -0.0051$; $r^2 = 0.9723$

Fig. 7. Standard curve and its equation of Nile blue LAS.

a) at 627nm



b) at 560 nm



$$ABS = K3C^3 + K2C^2 + K1C + K0$$

K1 = 0.0019

K0 = 0.0215

r^2 = 0.9998

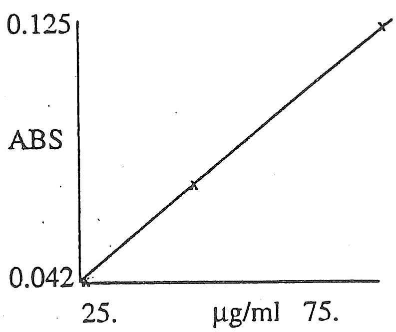
K1 = 0.0006

K0 = 0.0158

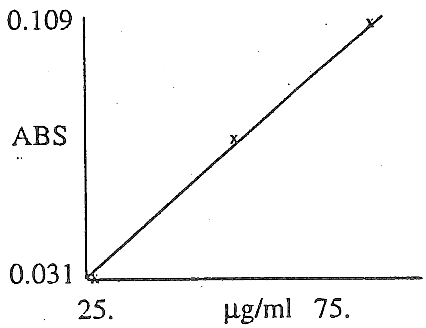
r^2 = 1.0000

Fig. 8.1. Standard curve and its equation of toluidine blue with LAS

a) at 655 nm



b)at 625 nm



$$ABS = K3C^3 + K2C^2 + K1C + K0$$

K1 = 0.0017

K0 = 0.0000

r^2 = 0.9998

K1 = 0.0016

K0 =-0.0053

r^2 = 0.9862

The LAS amounts found in seawater and 25μg LAS added seawater with methylene blue at α- and β- bands are shown in Table 2.

Table 2. LAS amount in seawater at α- and β- bands of methylene blue (μg/L).

Sample	α- band	β-band
Seawater	11.05	10.90
25μg LAS added seawater	35.90	35.93

Table 3. shows the LAS amounts by using azur I in seawater and Ömerli reservoir.

Table 3. LAS amount in seawater and Ömerli reservoir at α- and β- bands of azur I (μg/L).

Sample	α-band	β-band
Seawater	47	45
Ömerli reservoir	57	55

Table 4. shows the LAS amounts by using Nile blue in seawater and by dopping 25 μg/L of LAS for the control.

Table 4. LAS amounts in seawater and by dopping 25μg LAS at α- and β- bands of Nile blue (μg/L).

Sample	α-band	β-band
Seawater	11.40	10.93
25μg LAS added seawater	37.01	36.70

Table 5 shows the amount of LAS determined with toluidine blue at α- and β- bands.

Table 5. LAS amount in seawater at α- and β- band of toluidine blue (μg/L).

Sample	α-band	β-band
Seawater	43.9	43.2

Table 6. shows the results of Nile blue with barbiton buffer in seawater and LAS added seawater.

Table 6. The amount of LAS in seawater at α- and β- bands of Nile blue with barbiton buffer(μg/L) .

Sample	α-band	β-band
Seawater	43.9	43.2
50μg LAS added seawater	106.7	103.2

As can be seen in Table 2. the difference between the amounts of LAS determined with methylene blue at α- and β- bands in seawater is 0.15 μg/L considered as negligible. The corresponding amount for the seawater added of LAS is 0.03 μg/L.

The difference at α- and β- bands measurements of tested dyes: for azur I in seawater and Ömerli reservoir water are 2 μg/L (Table 3), for Nile blue at 627 and 560 nm as 0.47μg/L for seawater and 0.31μg/L for the LAS added sample, (Table 4), for Nile blue buffered at 625 nm and 580 nm are 0.7 μg/L in seawater and LAS added seawater 3.5 μg/L and for toluidine blue at 655 and 625 nm is 0.7μg/L.

Table 7 shows the comparison of methylene blue and Nile blue results.

Table 7. Comparison of methylene blue and Nile blue results (µg/L)

	Methylene blue		Nile blue	
	α- band	β- band	α- band	β- band
Sample				
Seawater	11.05	~~10.80~~	11.33	10.68
25µg LAS added seawater	35.90	35.83	37.00	36.71

The differences between the measurements for α- and β- bands are 0.28 µg/L and 0.12 µg/L respectively, in the case of 25 µg/L LAS added samples was found 1.1 µg/L at α- and 0.88 µg/L at β- band for methylene blue and Nile blue respectively. The difference in the results of both of the dyes are negligible, but methylene blue can be preferable.

In the earlier studies methylene blue and toluidine blue O (Güven *et al.*, 1994) and azur A and methylene blue (Akıncı and Güven, 1997) were used for metachromatic assay of anionic detergents in seawater. In this work additionally Nile blue, toluidine blue and azure I were examined as well in state of toluidine blue O and azur A.
In this study was found barbiton buffer has no influence on the assay.

According to the results, the determination of LAS were nearly similar at α- and β- bands in seawater and Ömerli reservoir.
The best results are obtained with methylene blue at α-band and β-bands, Nile blue being a close second.

This investigation showed that metachromatic method can be used to support MBAS method.

Özet

Standard Methods'a göre suda aniyonik yüzey aktif madde (LAS), metilen mavisi (MBAS) metodu ile tayin edilir. Bu çalışmada deniz suyunda LAS'ın tayini için metakromatik metod önerilmiştir. Bunun için kullanılan boyalar: metilen mavisi, azur I, Nile mavisi, toluidin mavisidir. Bu boyalar arasında en iyi sonucu metilen mavisi vermiştir. Nile mavisi ise buna çok yakın sonuçlar göstermiştir. Deniz suyu dışında su havzalarında (Ömerli havzası) LAS miktarı aynı metodla tayin edilmiştir. Bu tayinin kontrolü için ayrıca LAS ilave edilmiş deniz suyu ve Ömerli havzası sularında LAS miktarı sonuçları benzerdir. Bu çalışma sonunda metakromatik boyanın λ max.'ı yanında metakromatik band (β-band) ile de tayininin yapılabileceği gösterilmiştir. MBAS ve metakromatik tayin bulguları arasında ihmal edilebilecek oranda çok küçük fark olduğu olduğu saptanmıştır. Böylece metakromatik metodun LAS ın tayininde MBAS metodu yanında kullanılabileceği saptanmıştır.

Acknowledgement
The authors thank to LEVER Izmit for providing of LAS sample.

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Received : 20. 10. 2001
Accepted : 10. 12. 2001